



Transient Paralysis Associated with Marek's Disease Virus in a Poultry Flock

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ABSTRACT

Transient paralysis (TP) is a non-neoplastic neurological disorder in poultry caused by Marek's Disease Virus (MDV). It is characterized by temporary ataxia and paresis, primarily due to inflammatory damage in the central nervous system (CNS) rather than neoplastic transformation. The present study reported an outbreak of MDV-induced TP in a commercial 80,000 Lohmann LSL-Lite layer flock aged 95 days in Alborz Province, Iran, where affected hens exhibited sudden-onset paralysis and ataxia, with those that survived recovering spontaneously within three to five days without intervention. Although the flock had been vaccinated against MDV at hatch, neurological signs appeared at 95 days of age. Cerebellar samples were collected from ten clinically affected hens, including five collected postmortem from deceased chickens and five from live chickens that were humanely euthanized. Real-time polymerase chain reaction (PCR) confirmed infection with MDV field strains through strong amplification of pp38-Vir (1) and pp38-Vir (3) probes. In contrast, the CVI988 vaccine strain was not detected in samples collected at 95 days post-vaccination, which raised concerns regarding the quality of the vaccine and the proper administration of vaccination protocols. Histopathological examination of cerebellar tissues from three hens revealed vasogenic edema, perivascular cuffing, vasculitis, and widespread inflammatory cell infiltration. No evidence of tumor formation, mitotic activity, or neoplastic lymphoid proliferation was observed. These findings helped to distinguish TP from classical neoplastic Marek's Disease. The present study highlighted the need for early and accurate differentiation of TP from other neurological disorders, such as ionophore toxicity, botulism, and vitamin deficiencies, given its transient condition. The occurrence of TP in a vaccinated flock emphasizes the complexity of MDV pathogenesis, possible shortcomings in vaccine efficacy or its application practices, and the need for improved immunization strategies and biosecurity measures. Early molecular diagnostics and histopathological evaluation are essential for managing MDV-induced TP and mitigating its impact on poultry farms.

Keywords: Field strain, Histopathological examination, Marek's disease virus, Neurological disorder, Real-time PCR, Transient paralysis

INTRODUCTION

Transient paralysis (TP) is a neurological condition in poultry linked to Marek's Disease Virus (MDV), a highly contagious herpesvirus with oncogenic properties. In contrast to the classical neoplastic form of Marek's Disease (MD), which results in lymphoid tumor development, TP presents as temporary ataxia and paresis, most commonly involving the legs and wings. This condition results from inflammatory damage to the central nervous system (CNS), particularly the cerebellum, rather than neoplastic transformation (Abdul-Careem et al., 2006; Nair, 2018). Chickens typically recover within three to five days without intervention, distinguishing TP from the progressive, tumor-associated paralysis seen in classical MD (Swayne et al., 1989).

The pathogenesis of TP involves vasogenic edema, vasculitis, perivascular lymphocytic infiltration, and inflammation-induced vascular leakage, which disrupt normal neural function and cause transient neurological signs (Witter et al., 1999). The MDV strains responsible for TP are typically highly virulent (vvMDV) and very virulent plus (vv+MDV) strains, which exhibit strong neurotropism without necessarily inducing tumors (Kennedy et al., 2017). The presence of TP in both vaccinated and unvaccinated flocks suggests that immune response variability and exposure to virulent MDV field strains play a crucial role in disease manifestation (Mescolini et al., 2019).

Accurate diagnosis of TP is critical because its clinical signs can mimic other neurological disorders. Conditions such as ionophore toxicity (e.g., salinomycin poisoning), botulism, Newcastle Disease (ND), Avian Encephalomyelitis (AE), and vitamin E/Selenium deficiencies present with similar paralysis and ataxia (Parker and Schierman, 1983;

[Quevedo et al., 2022](#)). Molecular diagnostics methods such as polymerase chain reaction (PCR) and histopathological examination are essential for confirming MDV-induced TP. Classical MD is characterized by lymphoid neoplasia and tumor formation ([Mescolini et al., 2022](#)). In contrast, TP is a purely inflammatory condition without evidence of neoplastic proliferation, mitotic activity, or tumor development ([Swayne et al., 1989](#)).

Despite routine MDV vaccination, outbreaks of TP continue to be reported, highlighting concerns regarding vaccine efficacy or its application practices and the emergence of more virulent MDV field strains ([Gimeno et al., 1999](#)). Since TP typically affects young pullets before peak production, economic losses arise from reduced feed intake, handling costs, and potential losses due to misdiagnosis and inappropriate or unnecessary treatment ([Reddy et al., 2017](#); [Piriaei et al., 2023](#)). The present study documented an outbreak of MDV-induced TP in a commercial layer flock in Alborz province, Iran, emphasizing its clinical presentation, molecular confirmation, and histopathological findings. By differentiating TP from other neurological disorders, the present study aimed to highlight the importance of early detection, improved diagnostic accuracy, and optimized vaccination strategies to mitigate MDV-related neurological syndromes in poultry.

MATERIALS AND METHODS

Ethical approval

All procedures involving live chickens in the present study were conducted in accordance with the ethical guidelines and animal welfare standards of the Sana Institute for Avian Health and Diseases Research, Tehran, Iran.

Flock description and sample collection

The present study was conducted on a commercial poultry farm in Alborz province, Iran, which housed approximately 80,000 Lohmann LSL-Lite pullets aged 95 days distributed evenly across two poultry houses with 40,000 chickens in each. The flock was reared under a floor-rearing system and received a balanced diet formulated according to the Lohmann LSL-Lite management guide and NRC (1994) nutritional recommendations, with unrestricted access to feed and water ([Lohmann Tierzucht GmbH, 2016](#)). The chickens were part of a routine vaccination program, including vaccination against MDV (Table 1).

During the investigation, cerebellar tissue samples were collected from ten clinically affected Lohmann LSL-Lite pullets aged 95 days, including five that had died and five that were in poor clinical condition and were humanely euthanized using carbon dioxide (CO₂) gas. Euthanasia was performed by exposing the chickens to CO₂ in a sealed chamber following accepted animal welfare protocols ([Boyal et al., 2020](#)). As the cerebellum is the primary site affected in transient paralysis, tissue samples were collected postmortem to evaluate associated inflammatory and vascular changes. Additional fresh cerebellar tissues were snap-frozen and stored at -20°C for molecular diagnostics, ensuring sample integrity during transportation and analysis.

Molecular diagnosis

The cerebellar tissue samples were homogenized using a tissue homogenizer (Wiggins Company) to obtain a single pooled sample, ensuring a representative and uniform specimen for molecular analyses. According to the manufacturer's instructions, DNA was extracted from cerebellar tissues using the Kiagene Fanavar Tissue Kit (Kiagene Fanavar Company, Iran). The quality and concentration of the DNA were evaluated with a NanoDrop spectrophotometer to ensure its suitability for the next step of the study.

To identify and distinguish MDV strains, real-time PCR was utilized with primers and probes designed specifically for the *pp38* gene (Table 2). Different probes served distinct purposes, the *pp38*-CVI probe was used to detect vaccine strains, including CVI988, and some classical virulent strains. To enable comprehensive detection of MDV strains, the *pp38*-Generic probe was used, capable of identifying both vaccine-origin and virulent field isolates. In contrast, the *pp38*-Vir(1) and *pp38*-Vir(3) probes were specifically designed to detect virulent field strains, thereby facilitating distinction from vaccine strains ([Davidson et al., 2017](#)). For validation purposes, a sample containing the CVI988 vaccine strain was included as a positive control.

The PCR reactions were prepared in 20 µL volumes containing 10 µL of 2× TaqMan Universal PCR Master Mix, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL of probe (10 µM), 2 µL of DNA template, and 6 µL of nuclease-free water. The real-time PCR conditions included an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. Melting curve profiles were used to confirm amplification specificity. This evaluation ensured the PCR's specificity and efficiency, enabling accurate differentiation between virulent MDV field strains and vaccine-related strains ([Davidson et al., 2017](#)).

Table 1. The routine vaccination schedule was administered to the Lohmann LSL-Lite pullets during the rearing period (Hatchery to 16 weeks of age)

Age (Days)	Target diseases	Strains or Subtypes	Product name	Company and country	Route of administration
Hatchery	MD	HVT + Rispens	Marek VAC Bivalent Frozen	FATRO (Italy)	Subcutaneous injection
1	ND	PHY.LMV.42	VITABRON L	Ceva (Hungary)	Spray
	+ IB	+ H120			
	IB	1/96	Ibird	Ceva (Hungary)	Spray
9	IB	4/91	Nobilis IB 4/91	MSD (Spain)	Eye drop
	ND	Clone	VAXXON	IZO VAXXINOVA (Italy)	Eye drop
	ND + AI + IBD	Ulster 2C + H9N2 + VNJO	Gallimune	Boehringer Ingelheim (Italy)	Intramuscular Injection
	IB	Variant 2	Phivax IBV206	Phibro (Ireland)	Eye drop
13	IBD	D78	Nobilis Gumboro D78	MSD (Spain)	Drinking water
15	IBD	D78	Nobilis Gumboro D78	MSD (Spain)	Drinking water
19	ND + IB	Lasota + H120	VAXXON	IZO VAXXINOVA (Italy)	Drinking water
22	IBD	D78	Nobilis Gumboro D78	MSD (Spain)	Drinking water
30	IBD	D78	Nobilis Gumboro D78	MSD (Spain)	Drinking water
33	ND	Lasota	NEW L	Ceva (Hungary)	Drinking water
42	ND + IB	Lasota + H120	VAXXON	IZO VAXXINOVA (Italy)	Drinking water
45	AI	H5	DHN	Harbin Weike Biotechnology Co.Ltd (China)	Intramuscular injection
	ILT	PV/64	VAXXON ILT	IZO VAXXINOVA (Italy)	Eye drop
	ND	GVII	Medivac	Medion Farma (Indonesia)	Intramuscular injection
55	ND	Lasota	NEW L	Ceva (Hungary)	Drinking water
70	ND	Lasota	NEW L	Ceva (Hungary)	Drinking water
85	ND	Lasota	NEW L	Ceva (Hungary)	Drinking water

AI: Avian Influenza, IB: Infectious Bronchitis, IBD: Infectious Bursal Disease, ILT: Infectious Laryngotracheitis, MD: Marek's Disease, ND: Newcastle Disease

Table 2. Primers and probes used for Real-time PCR in the present study

Primer/Probe ID	Sequence (5'-3')	Modification	Reference
MD-forward	GAGCTAACCGGAGAGGGAGA	-	Davidson et al. (2017)
MD-reverse	CGCATACCGACTTTCGTCAA	-	
pp38-CVI Probe	CCCACCGTGACAGCC	FAM-BHQ1	
pp38-Vir (1) Probe	CCCACTGTGACAGCC	FAM-BHQ1	
pp38-Vir (3) Probe	CTCCCACTGTGACAGCC	FAM-BHQ1	

pp38-Generic probe	GCTACCGCCTGAGCC	FAM-BHQ1
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Histopathological examination

Cerebellar tissues were fixed in 10% neutral buffered formalin for 48 hours, then embedded in paraffin and sectioned at 5 µm thickness. The sections were placed on glass slides, deparaffinized, and stained with hematoxylin and eosin (H&E). Microscopic evaluation was carried out using a light microscope (Olympus CX23, Japan) to assess inflammatory and vascular lesions associated with MDV infection (Bancroft and Gamble, 2008).

RESULTS

Clinical findings

Out of a flock of 80,000 chickens, 390 cases developed transient neurological signs consistent with TP, including ataxia and paresis. These chickens exhibited temporary loss of coordination, difficulty in standing or walking, and limb weakness (Figure 1). Unlike progressive MD paralysis, 360 cases that survived recovered fully within three to five days without intervention. Although these chickens had trouble with movement, they showed no other signs of systemic illness. There was no fever, lethargy, or anything pointing to a serious problem. Total mortality was 30 chickens, likely resulting from their temporary paralysis, which impaired access to food and water. The rest of the flock appeared unaffected and had feed intake. They moved around as usual and showed no unusual behavior. This condition did not appear to have any lasting effects on the flock's overall health.

Molecular diagnosis results

Real-time PCR analysis confirmed the presence of MDV DNA in cerebellar tissues from the affected chickens. The positive control (vaccine sample) exhibited strong amplification with the pp38-CVI probe, showing a Ct value of 17.8 and a valid amplification curve, confirming the assay's reliability. The vaccine utilized was a bivalent MD vaccine containing HVT and Rispens CVI988 strains, produced by Vaxxinova GmbH (Italy).

The suspected samples did not amplify above the threshold with the pp38-CVI probe, ruling out the presence of vaccine-related MDV. However, strong amplification signals were observed with the pp38-Vir (1) and pp38-Vir (3) probes, indicating the presence of virulent MDV field strains. The Ct value for pp38-Vir (1) was 30.8, while pp38-Vir (3) amplified at 20.2, both with a distinct amplification curve, suggesting a high viral load of these virulent MDV strains. A vaccine sample containing the CVI988 strain (Vaxxinova GmbH, Italy) was used as a positive control in the PCR assay. Notably, this control did not amplify above the threshold with the pp38-Vir (1) and pp38-Vir (3) probes, confirming that the detected virus in the test samples was distinct from the vaccine-derived strain. The pp38-Generic probe detected MDV DNA in both vaccine and field strains, with Ct values of 19 (vaccine sample) and 28.7 (suspected sample), both with valid amplification curves, reinforcing the presence of MDV infection in the affected chickens. The negative control (nuclease-free water) demonstrated no amplification, confirming the absence of contamination or non-specific amplification in the assay. Melting curve analysis revealed clear, probe-specific peaks in both the suspected samples and the vaccine control. These results confirmed the specificity and accuracy of the real-time PCR reactions used for MDV strain differentiation. The results provided strong molecular evidence that the symptomatic chickens were infected with a virulent MDV field strain. The absence of amplification for the vaccine strain confirmed that the detected virus was not vaccine-derived. These findings demonstrated the effectiveness and specificity of the applied real-time PCR method for distinguishing field and vaccine strains.

Histopathological findings

Microscopic evaluation of cerebellar tissues from affected chickens indicated pathological changes consistent with TP caused by MDV. A prominent feature was perivascular cuffing, where lymphocytic infiltration surrounded small blood vessels, particularly in the cerebellar gray matter near Purkinje cells (Figure 2A). The inflammatory response was associated with vasogenic edema, characterized by fluid accumulation around blood vessels and within the surrounding neuropil (Figure 2B). These findings suggested disruption of the blood-brain barrier and vascular leakage due to inflammation. Additionally, vasculitis was observed, characterized by vascular inflammation and perivascular edema (Figure 2C, D), further supporting an immune-mediated pathogenesis. Higher magnification revealed dense inflammatory cell infiltration surrounding affected vessels (Figure 2E), reinforcing an inflammatory rather than a neoplastic process. Widespread microvascular edema affecting both medium-sized vessels and neuropil structures was also evident (Figure 2F, G), along with diffuse neuropil edema (Figure 2H), contributing to transient neurological dysfunction. Notably, there were no signs of neoplastic lymphoid proliferation, increased mitotic activity, or tumor formation in the examined tissues. This absence of neoplastic features differentiated the observed lesions from the neoplastic lesions typically seen in the classical lymphoproliferative form of MD. These findings strongly supported

MDV-induced TP as a non-neoplastic, inflammatory neuropathological condition rather than a tumor-associated disorder in affected chickens.



Figure 1. Clinical signs of transient paralysis in affected Lohmann LSL-Lite pullets aged 95 days

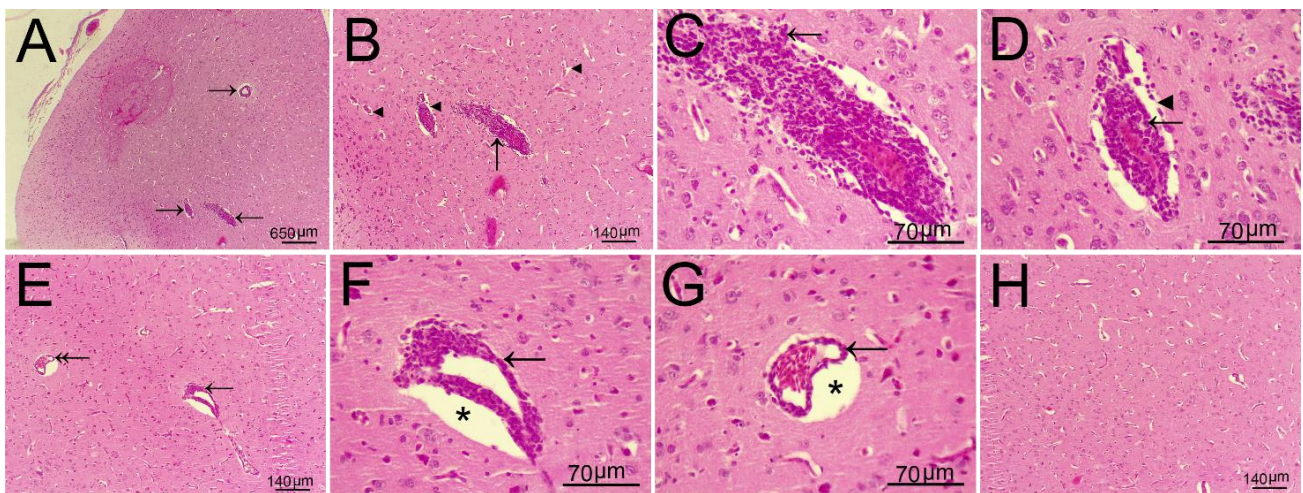


Figure 2. The cerebellum of Lohmann LSL-Lite pullets aged 95 days affected by Transient paralysis. **A:** Perivascular cuffing (Arrow) with prominent edema (Arrows) surrounding blood vessels and neuropil (H&E, $\times 200$), **B:** Dense inflammatory cell infiltration (Arrow) and perivascular edema (Arrowheads) around a large vessel (H&E, $\times 200$), **C and D:** Vasculitis (Arrow) with marked vascular inflammation and perivascular edema (double arrow, H&E, $\times 400$), **E:** Vasculitis (Arrow) with extensive edema around the affected vessel (double arrows head, H&E, $\times 400$), **F and G:** Edema surrounding microvasculature, medium-sized vessels (Asterisk), and neuropil (Arrow, H&E, $\times 200$), **H:** Diffuse edema affecting neuropil structures (H&E, $\times 400$).

DISCUSSION

Transient paralysis associated with MDV is rarely reported worldwide (Haems et al., 2023). In Iran, although classical MD is well recognized and recent molecular studies have identified virulent MDV-1 strains in commercial flocks, no reports have specifically described TP as a distinct clinical manifestation (Ghalyanchilangeroudi et al., 2022). Similarly, recent studies in China have reported highly virulent MDV strains causing visceral tumors and reduced vaccine efficacy, but the researchers in China did not document transient paralysis, highlighting the continued lack of explicit TP reports in field cases (Zhang et al., 2015). However, an experimental study indicated that around 18% of chickens infected with the RB1B strain developed TP signs, highlighting the neuroinvasive potential of virulent MDV strains and the need for targeted surveillance (Abdul-Careem et al., 2006).

MDV is widely recognized for its oncogenic potential, causing lymphoproliferative disease in poultry. However, it can also induce TP, a distinct non-neoplastic neurological syndrome. Unlike classical MD, which leads to tumor formation and progressive paralysis, TP results from acute inflammation, vasogenic edema, and immune-mediated vascular dysfunction affecting the CNS, particularly the cerebellum (Abdul-Careem et al., 2006). The present study provided evidence that TP can occur even in MDV-vaccinated flocks, raising concerns about vaccine efficacy or its application practices and the persistence of virulent MDV field strains in the study area. The neurological manifestations observed in this flock were consistent with MDV-induced TP, characterized by sudden-onset ataxia and paresis,

resolving within 3–5 days without permanent neurological deficits (Swayne *et al.*, 1989). These findings align with previous reports where TP resulted from MDV-induced inflammatory damage rather than neoplastic transformation (Mete *et al.*, 2016).

From an economic perspective, MDV-induced TP may not cause high mortality, but its impact is often underestimated. Temporary neurological dysfunction can disrupt production efficiency, reduce feed intake, and increase handling costs. More importantly, misdiagnosis-related treatments, such as unnecessary medication, nutritional supplementation, or antibiotic use, can lead to additional economic losses. Therefore, TP outbreaks highlight the need for improved vaccination protocols, enhanced biosecurity, and routine molecular surveillance of MDV field strains to mitigate economic risks in the field (Rozins *et al.*, 2019).

Histopathological analysis revealed perivascular lymphocytic infiltration, vasogenic edema, and vasculitis, supporting the inflammatory, immune-mediated nature of TP. The presence of edema in perivascular spaces and the neuropil suggests disruption of the blood-brain barrier, contributing to transient neurological dysfunction. The identification of vasculitis highlights vascular involvement in TP, differentiating it from classical MD. Diffuse neuropil edema suggests that TP results from temporary vascular dysfunction rather than permanent nerve damage. In contrast to the ongoing neurodegeneration characteristic of classical MD, TP resulted from acute, reversible inflammation of the vasculature. This condition improved as the inflammatory process diminished, allowing neurological function to recover (Spatz *et al.*, 2011).

The role of MDV virulence in TP pathogenesis remains a critical question. Real-time PCR analysis confirmed that the affected chickens were infected with MDV field strains, with strong amplification of pp38-Vir (1) and pp38-Vir (3) probes. These findings indicated that TP was caused by an actively circulating virulent MDV strain rather than a vaccine-related immunopathology (Mescolini *et al.*, 2019). Previous studies have shown that some MDV strains exhibit a high degree of neurotropism, replicating in CNS tissues and inducing inflammation without necessarily causing tumors (Quevedo *et al.*, 2022). The persistence of these virulent MDV strains despite vaccination underscores the need for continuous monitoring of evolving MDV pathogenicity (Parker and Schierman, 1983).

One of the biggest diagnostic challenges in poultry neurology is distinguishing MDV-induced TP from other causes of paralysis. Conditions such as ionophore toxicity (Salinomycin poisoning), botulism, Newcastle Disease (ND), Avian Encephalomyelitis (AE), and vitamin E/Selenium deficiencies can produce similar clinical signs, including ataxia and paralysis (Swayne *et al.*, 1989; Salvador *et al.*, 2021). Given its transient condition and spontaneous recovery, TP is often overlooked or misdiagnosed as a feed-related toxicity or an environmental factor (Mescolini *et al.*, 2022).

The occurrence of TP in a vaccinated flock raised epidemiological concerns. While vaccination reduces tumor formation and mortality, it does not fully prevent MDV infection, replication, or shedding (Bertzbach *et al.*, 2020). Transient paralysis cases suggest that certain MDV strains may still induce neurological disease, potentially due to immune escape mechanisms or inadequate vaccine-induced protection (Parker and Schierman, 1983; Nair, 2018). The emergence of virulent MDV strains capable of overcoming vaccine immunity remains a significant threat to poultry health and productivity (Abdul-Careem *et al.*, 2006). In addition to viral evolution, field vaccine failure may result from low-quality vaccines, cold chain issues, economic sanctions against Iran, and improper administration, such as the use of half doses by farmers. These factors together reduce the overall effectiveness of MD vaccination in commercial settings.

CONCLUSION

The present study confirmed that TP is a distinct neurological condition caused by infection with virulent field strains of MDV. Unlike the classical form of MD, which involves lymphoid tumor formation and irreversible paralysis, TP is driven by immune-mediated inflammation and reversible vascular disturbances in the central nervous system. Despite vaccination, TP outbreaks continue to occur, emphasizing the need for improved immunization strategies, strict biosecurity measures, proper vaccine handling and administration, and regular MDV strain monitoring, especially in light of potential limitations in vaccine quality and availability under field conditions. Early and accurate differentiation of TP from other neurological disorders is essential to ensure proper disease management, avoid misdiagnosis, and minimize economic losses in poultry production. Future research should focus on understanding MDV neurotropism, optimizing vaccines, and examining the role of host immunity in MDV-induced neurological syndromes.

DECLARATIONS

Authors' contributions

Jamshid Razmyar conceptualized, designed, and supervised the study. Field sampling and data collection were performed by Mohammad Barari and Mohammad Reza Danaeifard. Mohammad Hossein Nazem Shirazi carried out

laboratory experiments and molecular diagnostics. Omid Dezfoulan and Farhang Sasani performed histopathological examination and interpretation. The original draft of the manuscript was written by Mohammad Barari. All authors contributed to reviewing and editing the manuscript and read and approved the final version of the manuscript.

Ethical considerations

All procedures performed in this study were conducted ethically and responsibly, fully adhering to applicable codes of experimentation, relevant legislation, and compliance with ethical guidelines for animal welfare.

Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interests

The authors declared no conflicts of interest.

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